THE USE OF SALT TO ASSIST ENZYMATIC UNHAIRING OF FRESH HIDES, WITH HISTOCHEMICAL OBSERVATIONS*

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ABSTRACT

Pretreatment with dilute salt solution had been found advantageous in hastening the unhairing of fresh hides with enzyme. A series of controlled tests was performed to elucidate this relationship between salt concentration in the soak solution and the subsequent rate of unhairing, utilizing a numerical scoring system to interpret the data. The effect of frozen storage of fresh hide was also evaluated in the same manner. Results indicated somewhat different concentration optima for salt treatment, depending upon whether or not the hide had been frozen. Among four staining methods applied to thin sections of salt-treated hide the PAS stain successfully demonstrated changes induced in the basement membrane, while a metachromatic stain made other nearby structures visible.

INTRODUCTION

One of the most common methods of preserving animal skins is treatment with salt. Such treatment prevents the growth of many microorganisms which would otherwise attack and destroy the tissues. Salt treatment also serves other functions. The globular proteins, unwanted for leather-making are solubilized and partially removed. In a series of papers Kritzinger (8–12) reported his studies on the globular proteins of animal skins. He found (10) that 10% sodium chloride was the optimum concentration for removing globular proteins from animal skin and that the concentration of sodium chloride had a marked effect upon the composition of the nitrogenous material extracted. Roddy (18) found that salt extraction of coagulable proteins from a hide or skin while it is still in a freshly flayed condition improves the leather-making properties. Buechler and Lollar (1) used 5% sodium chloride at refrigerator temperature to remove the hair and epidermis from bullhide. By this treatment the entire epidermis could be

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taken off in sheets. Cordon (4) found that certain enzymes would rapidly loosen the hair on salt-cured hides but not on fresh hides. This was contrary to the findings of Burton, Reed, and Flint (2). Cordon also found that treatment of fresh hide with dilute sodium chloride made it subject to the action of the hair-loosening enzymes. This paper presents an attempt to evaluate this action of salt solutions by a series of controlled tests and to determine by means of histochemical observations the nature of the changes produced.

MATERIALS AND METHODS

Hides.—The hides used in these studies were heavy steers obtained from a local slaughterhouse shortly after flaying. Hide A, purchased in March, was used for preliminary unhairing tests and for histological study after soaking. Hides B and C, acquired in September and October, respectively, were used entirely for obtaining the unhairing data reported below. All hides were washed with water in a drum, and portions were fleshed and placed in a deep-freeze chest to be thawed as needed. Other similar portions were refrigerated at 34°F. and used within a few days without freezing. Three-inch squares were cut from each hide for the experimental tests.

Enzyme.—The enzyme used was HT Concentrate, produced by the Takamine Laboratories* from a thermophilic bacterium. Its proteolytic and amylolytic potencies as related to its hair-loosening ability have recently been reported (5). Unless otherwise stated it was used at a concentration of 0.1%.

Hide treatments.—Soaking was carried out at room temperature in covered dishes. Hide pieces were completely immersed in solutions containing various concentrations of sodium chloride. For convenience the salt solutions were made up by adding predetermined amounts of salt to one-liter volumes of water, then the concentrations were calculated as grams per 100 grams of solution. Duplicate pieces were removed at daily intervals, rinsed briefly, and placed in 100 ml. enzyme solution held in covered containers in a constant-temperature water bath at 45°C., unless otherwise stated. Phenyl mercuric acetate (0.15 g. per l. solution) was used throughout, both in the soaks and enzyme solutions, to inhibit bacterial growth. At this concentration the compound does not seem to interfere with enzyme action. To obtain maximum protection during prolonged incubation the solution should be replaced every day.

Estimation of hair looseness.—The hide pieces were tested for degree of hair looseness at four 12-hour intervals during incubation by means of a scraping device designed for this purpose (6). The blade was drawn

*The mention of trade names or companies does not constitute an endorsement by the Department of Agriculture over other products of a similar nature not mentioned.

across the hide under constant load in the direction of hair pattern. After ten strokes or less, the approximate percentage of scraped area cleared of hair was estimated visually. Thus a reading of 5-90 means that five strokes removed 90% of the hair and that further attempts were ineffective. Readings of + or ++ indicate slight looseness, as detected by plucking with the fingers, below the threshold of this method. When hair looseness was incomplete (readings poorer than 3-95), the hide pieces were returned to their enzyme solutions for an additional 12 hours and then retested. When adequate loosening was achieved, the pieces were discarded but were scored as complete for any remaining intervals.

Preparation of sections.—Narrow strips were cut from each hide piece after soaking, and placed in 10% formalin (1:10 dilution of U.S.P. formaldehyde solution) for at least two days at room temperature for fixation. Those to be studied were then sectioned vertically on a freezing microtome at 30 to 40 microns. Sections were stored in 50% ethanol to remove formalin and minimize water extraction of soluble components.

Staining methods.—Four different methods were applied routinely to all sections in an attempt to detect changes due to treatment.

1. Periodic acid–Schiff stain (PAS) to demonstrate polysaccharide-containing material. The procedure of Hotchkiss (7) using alcoholic reagents was found preferable. This method, with suitable controls, is said (17) to be specific for 1, 2-glycols or amino-glycols and certain lipoids, which are oxidized to dialdehyde by periodic acid and made visible with Schiff reagent.

2. Crystal violet stain to show globular proteins. This was essentially the method used by Roddy (18) in his study of coagulable proteins. Diffusion of the stain occurs rather rapidly, so that slides must be examined or photographed within one day to give valid results.

3. Toluidine blue to stain nuclei orthochromatically (blue to purple) and acid mucopolysaccharides metachromatically (pink to red). Dilute dye was used (15) in MacIlvaine buffers at pH 2, 4, 6, and 8. Sections were then rinsed rapidly, dehydrated, cleared, and mounted in balsam. Several good reviews (17, 19) are available to explain the theory of metachromasia and to help interpret results.

4. Alcian blue–PAS stain to show differentially various mucins and polysaccharides. The procedure was basically according to Steedman (13, p. 287) as modified by Mowry (16). Acidified dye was used, followed by alkaline fixation and then the complete PAS procedure. The blue and red colors provide a very effective contrast.

RESULTS AND DISCUSSION

In previous studies on the effect of salt treatment of fresh hide on hair loosening by enzymes, it had been consistently found that preliminary soaking in 2% to 8% NaCl was necessary to permit proper loosening with enzyme. If the hide was unsoaked, or soaked in plain water or strong salt, it exhibited considerable resistance to enzyme action. Also it was confirmed that dilute salt solution had a slow unhairing action by itself (3 to 5 days). In this case the hair-loosening action was too slow for commercial application but was of interest because almost the entire epidermal system could be removed intact. Figure 1 shows a photograph of the underside of this epidermal layer.

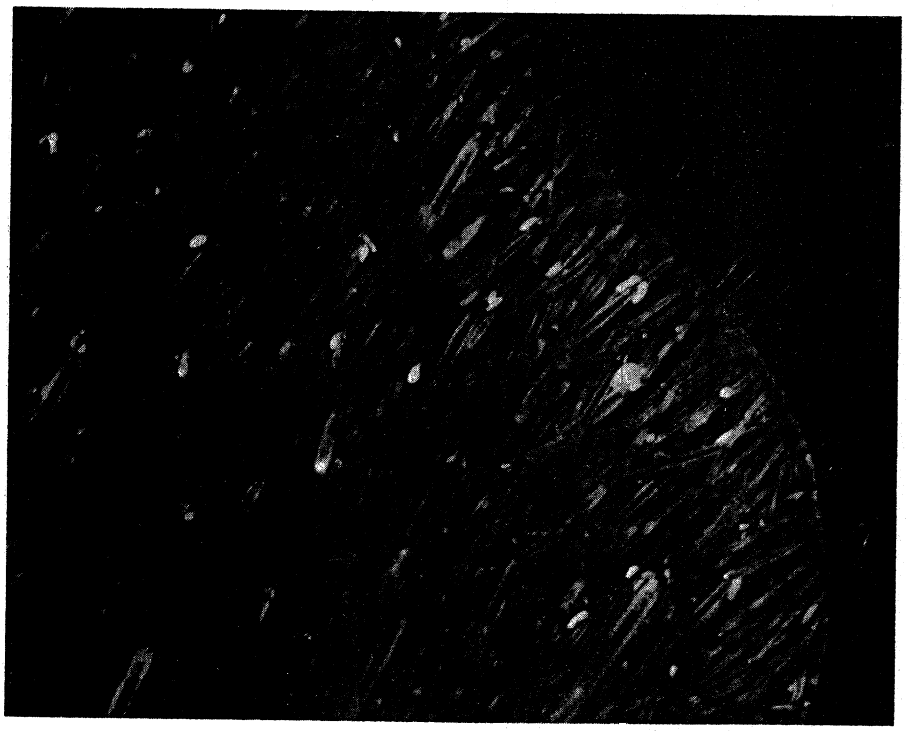

FIGURE 1.—Enlarged view of epidermis, with hairs and glands attached, removed from frozen hide after soaking for 5 days in 5% salt solution.

All of this work had been done with fresh hide held in the frozen state for varying periods. Preliminary experiments for this report employed hide that had been held frozen for over six months, using salt soaks over the range 0% to 20%. With this material the enzymatic unhairing was uniformly rapid in each case, showing no difference whatever due to pretreat-

ment. Since no other reasonable explanation could be found, it was deduced that there might be a definite effect on the hide due to frozen storage that could alter its response to enzyme. In a recent study Cooper and Sykes (3) have shown that freeze-drying of fresh hide causes irreversible changes which prevent complete rehydration of the hide, with evidence of denaturation of both globular proteins and mucopolysaccharides. Consequently three additional fresh hides were obtained over a period of time to test separately the effects of freezing and salt-soaking on the rate of enzymatic unhairing.

Tests with fresh nonfrozen hides.—No unhairing data are presented here for hide A since only a few replicates were run. However, two other hides (referred to elsewhere as hide B and hide C) were compared in several ways, both as nonfrozen and frozen material. Table I illustrates a representative experiment to determine the relative effectiveness of pretreatment with various levels of salt in enhancing the rate of unhairing by 0.1% solutions of enzyme. This concentration of enzyme was purposely rather weak in order to bring out differences between treatments. The scoring system, shown in the last two columns, was derived as follows: An arbitrary end point for readings of hair looseness (3–95) was chosen to represent a degree of looseness just short of absolute completion (1–100). Whenever this end point or a reading closer to absolute completion was reached during examination of a hide piece, the letter X was added to the reading recorded in one of the four center columns, to denote scoring of one point. The hide piece was then discarded but was still scored as completed for any remaining time intervals. By adding these points horizontally for each pair of duplicates one obtains the numerator for the fraction recorded in the next to the last column. The denominator is merely the maximum possible score. Note that this denominator when divided by four indicates the number of replicates involved. The last column expresses this fraction as a whole number for comparative purposes.

Table II summarizes the data for both hides obtained from a number of similar tests. Because of the large variability between hides as well as between pieces of the same hide the composite scores gave the best picture of the effect of treatment with solutions of varying salt concentrations. It can be seen that soaking for two days was always a little better than for one day. Data were also recorded after three days (and sometimes after four and five days) but did not seem significantly different and consequently are not shown. Also there was a pronounced peak in activity after soaking in 4.8% salt, which confirmed previous experience (4). However, this peak did not recede much with increase in salt concentration, but rather a secondary higher peak was shown at the saturation point. This result was quite unexpected. Controls without enzyme consistently showed negligible looseness, whether incubated in water at 45 °C. or in the various salt solutions at room temperature.

TABLE I

REPRESENTATIVE EXPERIMENT SHOWING EFFECTS OF SOAKING IN SALT SOLUTIONS ON RATE OF ENZYMATIC UNHAIRING OF NONFROZEN FRESH HIDE

Soak Conditions		Hair Looseness Readings, 45°C				Unhairing Results	
NaCl	Days	12 hr.	24 hr.	36 hr.	48 hr.	Score	Score × 100
%		P* R†	P R	P R	P R		
0	1	0‡	+–++	10–90	3–98X		
		0	+–++	10–80	3–98X	2/8	25
	2	+–++	3–98X**	X	X		
		0–+	5–95	3–98X	X	5/8	63
1.2	1	++	5–95	3–98X	X		
		10–60	6–98	3–100X	X	4/8	50
	2	0–+	10–90	2–98X	X		
		++	7–95	3–98X	X	4/8	50
2.4	1	10–80	5–98	3–98X	X		
		++	7–95	3–98X	X	4/8	50
	2	0–+	3–98X	X	X		
		++	6–95	3–100X	X	5/8	63
4.8	1	10–70	3–98X	X	X		
		10–80	3–98X	X	X	6/8	75
	2	++	3–98X	X	X		
		++	5–98	3–98X	X	5/8	63
9.1	1	+–++	5–95	3–98X	X		
		+–++	5–90	3–98X	X	4/8	50
	2	7–98	4–100X	X	X		
		6–98	3–100X	X	X	6/8	75
16.7	1	+	5–98	3–98X	X		
		+–++	4–98	3–98X	X	4/8	50
	2	0	5–95	3–98X	X		
		4–95	4–100X	X	X	5/8	63
25.9	1	+	4–98	3–98X	X		
		+–++	3–98X	X	X	5/8	63
	2	0	3–98X	X	X		
		0	3–98X	X	X	6/8	75
No Soak		0	++	6–98	3–98X		
		++	6–95	3–98X	X	3/8	38
No Enzyme		0	0	0	+		
		0	0	0	0	0/8	0

*Number of pulls with scraper (*see* Methods section)
†Estimated percent of hair removed from scraped area
‡0 = hair tight; + and ++ = slight looseness not measurable by scraper.
**Symbol X denotes point scored for reaching end point (3–95 or better.) Add points horizontally for each duplicate pair to obtain score numerator; denominator is maximum number possible.

Effect of lower temperature. In a series of similar tests the incubation temperature for enzyme treatment was lowered from 45° to 40°C. No significant change was seen in the unhairing scores obtained; all values were somewhat lower but within about 7% of those shown in Table II.

TABLE II

SUMMARY OF UNHAIRING* SCORES† FOR NONFROZEN PORTIONS OF TWO FRESH HIDES

Soak Conditions		Hide B		Hide C		Composite		
% NaCl	No. Days	Score	Score × 100	Score	Score × 100	Score	Score × 100	Average
0	1	7/12	58	6/16	38	13/38	46	52
	2	6/12	50	10/16	63	16/28	57	
1.2	1	6/12	50	9/16	56	15/28	54	56
	2	7/12	58	9/16	56	16/28	57	
2.4	1	6/12	50	9/16	56	15/28	54	63
	2	9/12	75	11/16	69	20/28	71	
4.8	1	20/28	71	10/16	63	30/44	68	70
	2	9/12	75	11/16	69	20/28	71	
9.1	1	6/12	50	10/16	63	16/28	57	66
	2	9/12	75	12/16	75	21/28	75	
16.7	1	8/12	67	9/16	56	17/28	61	66
	2	8/12	67	12/16	75	20/28	71	
25.9	1	16/20	80	11/16	69	27/36	75	82
	2	16/16	100	12/16	75	28/32	88	
No Soak	–	6/12	50	7/16	44	13/28	46	
No Enzyme	–	0/12	0	0/16	0	0/28	0	

*Treated with 0.1% HT Concentrate at 45° C.
†Scoring system explained by Table I.

TABLE III

SUMMARY OF UNHAIRING* SCORES† FOR FROZEN PORTIONS OF TWO FRESH HIDES

Soak Conditions		Hide B		Hide C		Composite		
% NaCl	No. Days	Score	Score × 100	Score	Score × 100	Score	Score × 100	Average
0	1	21/28	75	9/16	56	30/44	68	67
	2	12/16	75	9/16	56	21/32	66	
1.2	1	21/28	75	9/16	56	30/44	68	70
	2	13/16	81	10/16	63	23/32	72	
2.4	1	21/28	75	9/16	56	30/44	68	70
	2	13/16	81	10/16	63	23/32	72	
4.8	1	20/28	71	9/16	56	29/44	66	74
	2	15/16	94	11/16	69	26/32	81	
9.1	1	25/28	89	11/16	69	36/44	82	82
	2	16/16	100	10/16	63	26/32	81	
16.7	1	28/28	100	11/16	69	39/44	89	87
	2	15/16	94	12/16	75	27/32	84	
25.9	1	22/24	92	11/16	69	33/40	83	83
	2	3/8	100	12/16	75	20/24	83	
No Soak	–	26/36	72	9/16	56	35/52	67	
No Enzyme	–	0/24	0	0/16	0	0/40	0	

Effect of adding salt to enzyme solutions. It was thought that perhaps the time required for soaking could be eliminated by adding low levels of salt directly to the enzyme solution and performing both functions simultaneously. A number of concentrations, from 0% to 20%, were tried in several tests with both hides at 45°C. In general the scores remained at least 10% below the first peak of activity shown in Table II; therefore this procedure was not as effective as preliminary soaking. In fact there was strong inhibition of activity with increasing salt content, starting at about 5%. Some evidence of weak stimulation was apparent with 1% NaCl (normal physiological level in living tissue). Controls without enzyme began to show very slight hair loosening in four days with salt concentration below 5%, but none above this point.

Tests with hides preserved by freezing.—The data summarized in Table III and which may be compared directly with Table II were obtained by using portions of hides B and C that had been held frozen for periods up to one month. It is apparent that in this series there was a much greater difference between hides than was noted before, since hide C consistently showed lower scores than hide B. Also there was very little, if any, advantage in soaking for two days rather than one. Results after soaking for three days, not shown, were more typical in giving higher scores for each treatment. It should be noted here that after soaking for two, and especially for three days, in the three weakest salt solutions, it was common to observe appreciable hair looseness (++) with frozen hide. This did not occur with the hide which had not been frozen. The average unhairing scores from Tables II and III were plotted for comparison in Fig. 2. Here it can be seen that hide which had been frozen always unhaired a little faster than that which had not been frozen. Also the two activity peaks in the curve for nonfrozen hide are clearly evident, while the curve for frozen hide has just one peak, between 10% and 20% salt. As time of frozen storage is extended this latter curve tends to level off. In seeking an explanation for this beneficial action of frozen storage it might be considered as another manifestation of the salt effect. Lovelock and others (14, and following papers in the same issue) have pointed out that during slow freezing of a tissue there is a gradual increase in the formation of ice crystals in the intercellular spaces. This removal of water results in a corresponding increase in the concentration of electrolytes dissolved in tissue fluids. It is conceivable that the concentration could reach effective levels and that the effect could increase with time.

Effect of lower temperature. In a series of similar tests the incubation temperature was changed from 45° to 40°C. as described for nonfrozen hide. Again there was no significant change in scores due to change in temperature, and the general response to conditions of soaking remained the same.

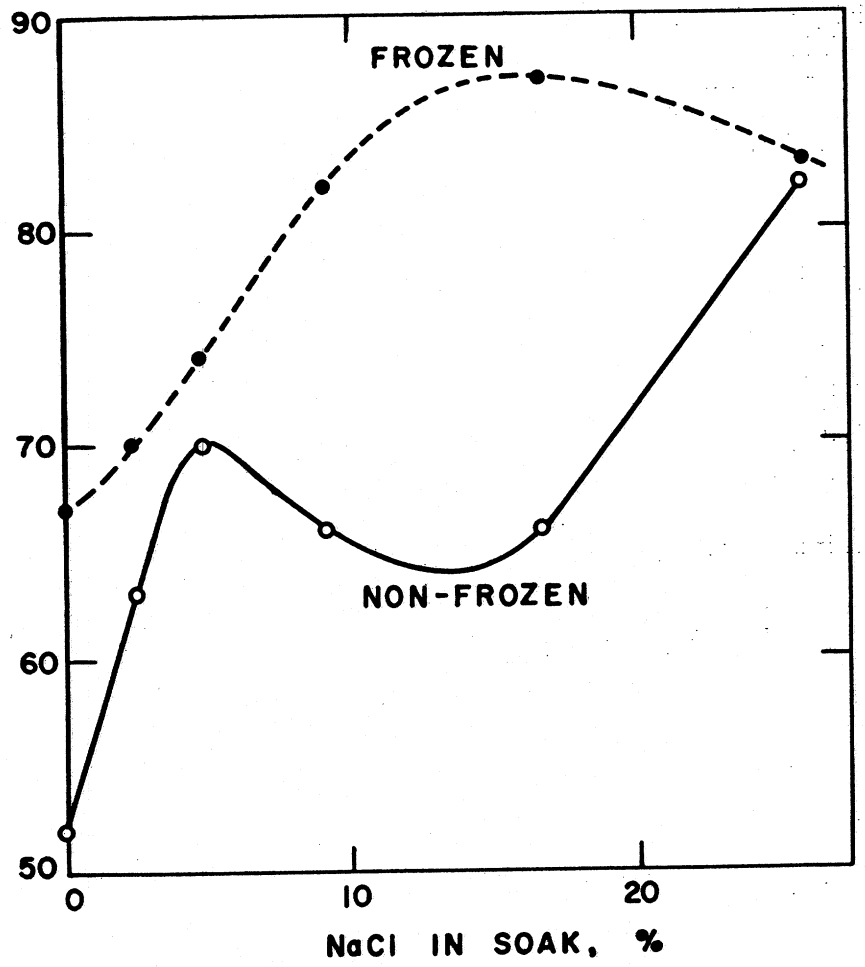

FIGURE 2.—Influence of salt concentration in the soak on the rate of unhairing of frozen and nonfrozen cattlehides with 0.1% HT Concentrate at 45° C.

Effect of stronger enzyme. In a short series of tests 0.2% enzyme was substituted for 0.1% at 45°C. Unhairing scores averaged about 10% higher in almost every instance but otherwise showed the same general response to soaking treatment.

Effect of adding salt to enzyme. A series of tests with various levels of salt added directly to the enzyme solutions was carried out as described above for nonfrozen hide. Again the frozen hide behaved in a similar manner to that which had not been frozen—there was weak stimulation at around 1% NaCl (however, the best score was more than 10% below the first ac-

tivity peak shown in Table III), and the same pattern of inhibition resulted with higher levels of salt. When 0.2% enzyme was used in place of 0.1%, the same general results were obtained except that the increase in inhibition was more gradual and did not become pronounced until about 9% salt concentration.

HISTOLOGICAL AND HISTOCHEMICAL STUDIES

In order to obtain additional information on the changes induced in hide components by salt treatment, sections from soaked nonfrozen hide pieces were subjected to various histochemical procedures and examined microscopically. Also an attempt was made to record some of the results photographically for reference purposes. It is understandably difficult to render in black and white the many shades and hues of a stained tissue and to achieve good focus with relatively thick sections. Therefore it is apparent that more information was gained from direct microscopic examination than can be shown in black and white prints. Nevertheless a few photos are included to help illustrate the results.

PAS stain.—It was expected that the PAS stain might prove the most informative, since it makes visible polysaccharide-containing materials including mucopolysaccharides, mucoproteins, and glyco-proteins. One or more of such components are known to be present in the grain layer and might well be the principal substrate concerned with hair loosening. When fresh, untreated hide was stained by this method (Fig. 3), the most prominent structure visible was an intense red band, of variable thickness depending upon the orientation of the cutting plane, immediately below the base of the epidermis. This is commonly referred to as the basement membrane, but the stained band probably includes more than the membrane proper. It appears to include also the uppermost zone of collagenous dermis, so modified as to contain a cementing substance, rich in polysaccharide, for holding the epidermis in place by means of minute extensions of the basal cells. Muscular tissue was also strongly stained, while a lighter shade appeared in the epidermal cytoplasm, the background of dermis (ground substance), and the thicker elastin fibers. It had been observed that treatment of hide with HT Concentrate removed practically all trace of the bright red band. Therefore, it seemed logical to examine this structure for any changes brought about by soaking in various salt solutions, since these treatments were shown to affect the rate of unhairing. Soaking in water (Fig. 4) did not seem to alter the appearance of the basement membrane, although the background staining in both dermis and epidermis was less intense. However, after the hide was soaked in 4.8% salt (Fig. 5), the picture was quite different. The stained band in the dermis was broader and much less intense, while the basal layer of epidermis remained rather dark, and there was gross separation between

FIGURE 3

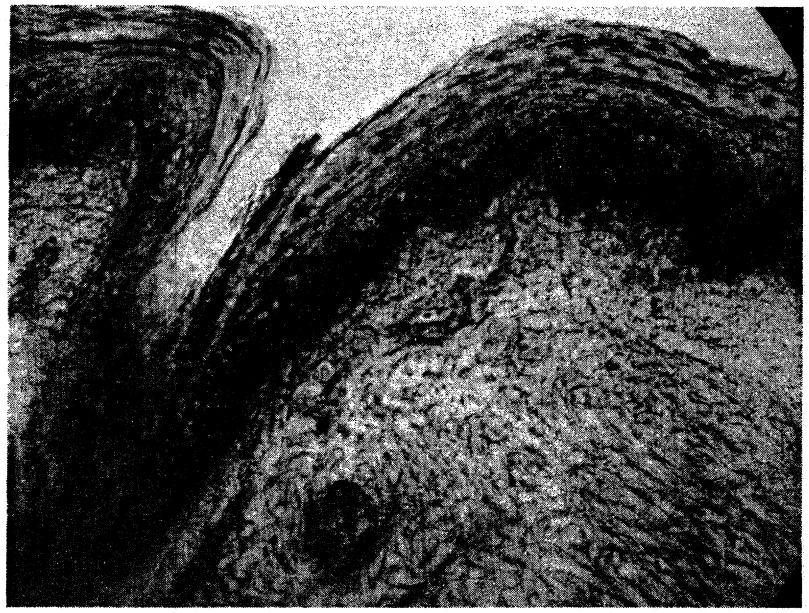

FIGURE 4

FIGURES 3–4.—Cross sections, nonfrozen fresh hide, PAS stain, green filter. ×200
Figure 3.—Untreated. Figure 4.—Soaked in water.

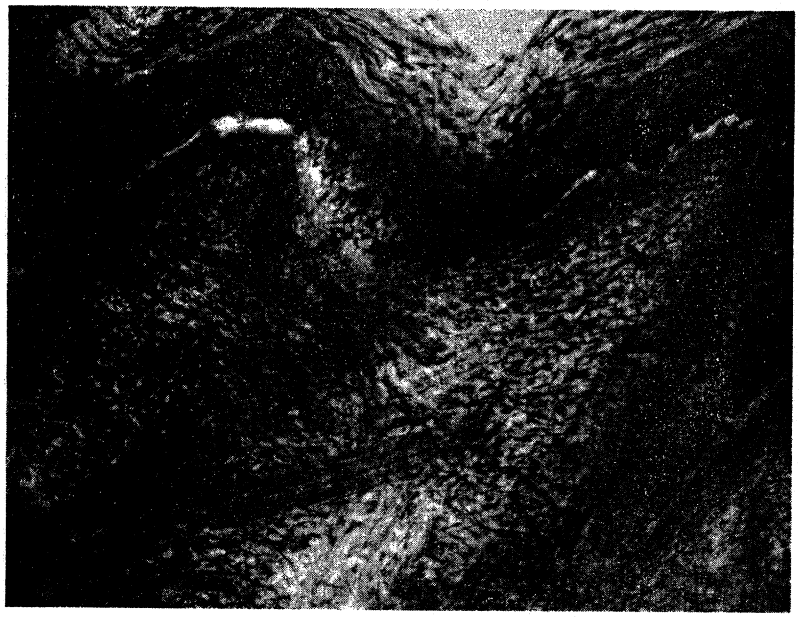

FIGURE 5

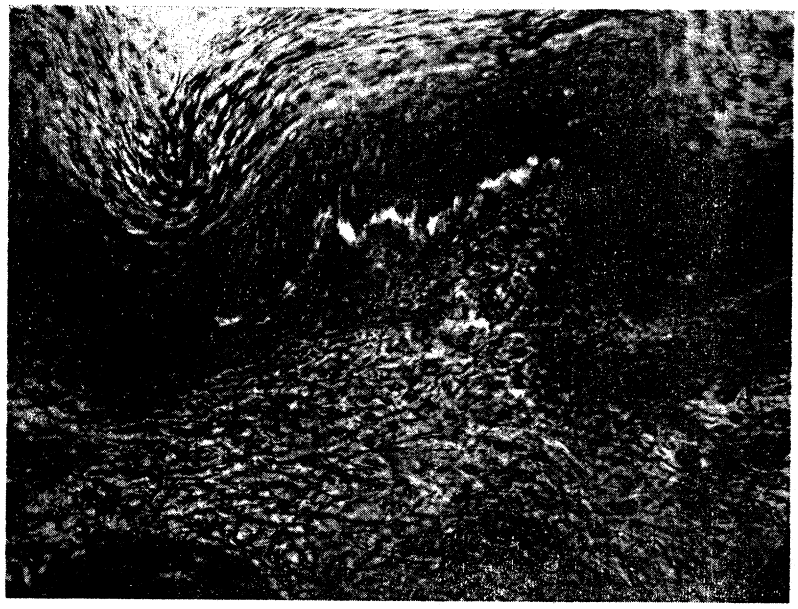

FIGURE 6

FIGURES 5–6.—Cross sections, nonfrozen fresh hide, PAS stain, green filter. ×200.
Figure 5.—Soaked in 4.8% salt. Figure 6.—Soaked in 9.1% salt.

FIGURE 7

FIGURE 8

FIGURES 7–8.—Cross sections, nonfrozen fresh hide, crystal violet stain, yellow filter. ×100. Figure 7.—Untreated. Figure 8.—Soaked in 4.8% salt.

FIGURE 9

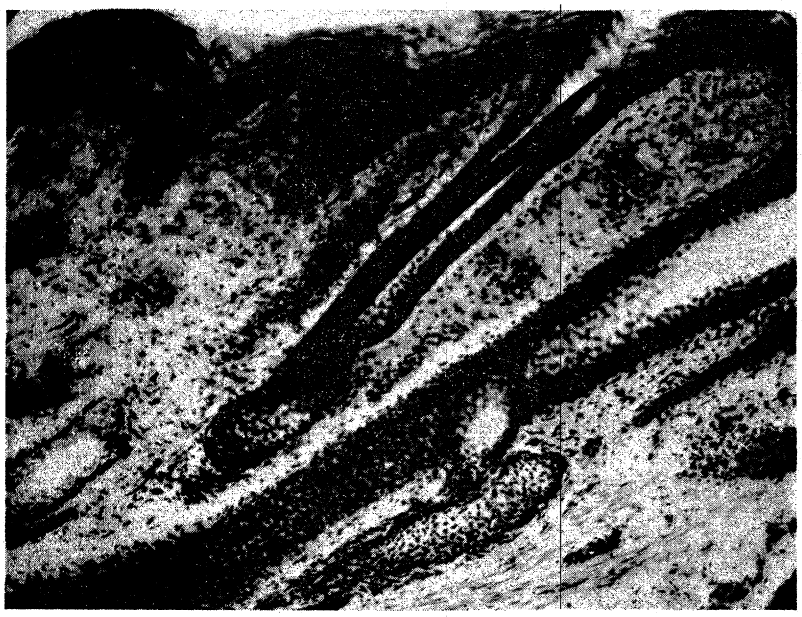

FIGURE 10

FIGURES 9–10.—Sections of nonfrozen fresh hide, stained with toluidine blue at pH 6, green filter. ×100. Figure 9.—Untreated. Figure 10.—Soaked in 4.8% salt.

the layers in many places. Similar changes, to a lesser degree, were noted after the hide was soaked in 2.4% salt. This apparently represents an early stage of unhairing, which is then brought to rapid completion by the enzyme. Soaking the hide in 9.1% salt (Fig. 6) gave a similar result (and a somewhat clearer photograph), although epidermal separation is not usually as extensive with strong salt solutions as it is with dilute ones. Note the abnormally thick epidermis in this sample—a frequent observation with this hide.

Crystal violet.—Some effects of pretreatment were also detected with the crystal violet stain, which was intended to make visible globular proteins. Freshly stained untreated hide (Fig. 7) showed intense coloration of all cellular elements such as epidermis, glands, blood vessels, and muscles. In addition there was moderate background staining throughout the grain layer but much weaker in the corium. Soaking the hide in water caused a pronounced reduction of stain intensity, especially in the epidermis. The weaker salt solutions (Fig. 8) did not seem to affect the cellular staining to any appreciable extent. However, there was a gradual but progressive reduction in the staining of the background of the grain as the salt content increased, while the corium became completely devoid of staining materials. The maximal effect appeared to be reached at 9.1% salt concentration, since a slight reversal occurred at the highest salt levels. Although these results showed no direct correlation with subsequent unhairing behavior, they do indicate that globular proteins are not prominent in the basement membrane but probably are more important in relation to tanning operations.

Metachromatic stain.—Staining with metachromatic dyes such as toluidine blue produced some striking color contrasts in hide sections. In fresh untreated hide the cell nuclei, and to some extent the cytoplasm of growing epidermis, appeared purplish blue, the natural color of the dye. However, in certain characteristic locations there were variable amounts of material that stained metachromatically in shades of pink to red. Staining at pH 4 to 6 was optimal for this purpose. The most noticeable locations where this appeared were the loose connective tissue around the capillary network of the upper grain layer, the sebaceous glands, the lower half of growing follicles, and the papillae of growing hairs. Also, in specialized cells of the upper grain layer called mast cells, the cytoplasm was filled with red metachromatic granules. Collagen and elastic tissue remained unstained, and the *stratum corneum* or top dead layer of epidermis was pale green. A section of the untreated fresh hide stained by this method at pH 6 is shown in Fig. 9; however, the pink and red areas are represented in this photograph by corresponding tones of medium to dark gray. Mast cells are the large dark spots scattered through the upper dermis. Montagna *et al* (15)

have shown some excellent color plates of metachromasia in growing hair follicles, which they associate with the process of keratinization.

When hide pieces had been soaked in dilute salt solutions (Fig. 10), there appeared to be no change in the metachromatic materials of the dermis, but the delicate violet shade (apparently due to both blue- and pink-staining elements) in the lower epidermis was lost. Soaking in water or strong salt caused osmotic disruption of the mast cells. Also it seemed that treatment with 9.1% salt or stronger reduced the intensity of the pink zones of the capillary beds. After treatment with the unhairing enzyme the tissues became devoid of metachromatic staining material. These findings indicate that the basement membrane apparently contains no sulfated mucopolysaccharides, but such materials are removed from the other tissues by the unhairing enzyme.

Alcian blue–PAS.—Alcian blue was also used to demonstrate mucins or mucoids. It was found to be specific for the same structures stained by toluidine blue but without metachromatic properties, since it produced only shades of blue. The color formed, however, is very resistant to chemical reagents, which permits counterstaining by procedures such as PAS. Although it has yielded no new information, it served to confirm results obtained with the other staining procedures.

SUMMARY

Results have been presented from a number of experiments to determine the relative effectiveness of various salt pretreatments in stimulating the rate of unhairing of fresh hides with a commercial enzyme. Parallel tests were also included to evaluate the additional factor of frozen storage of the hide. It was found that frozen hide always unhaired faster than nonfrozen hide after the same pretreatments. Plotting the rate of unhairing as a function of salt concentration in the soak solutions revealed that nonfrozen hide responded with two activity peaks, the first at about 5% salt and the second, a stronger one, at about 26% salt (saturated). With frozen hide there was only one broad peak, between 9 and 26% salt. Soaking the hide for two or three days caused faster unhairing than soaking for one day. There was no definite advantage in adding salt to the enzyme solution in place of preliminary soaking in salt solution, nor was there any real difference in results whether the enzyme solution was incubated at 40° or 45°C.

Histochemical evidence suggested several possible effects of salt pretreatment. The PAS stain, broadly specific for polysaccharide components, demonstrated a prominent structure referred to as the basement membrane (junction of dermis and epidermis) which is apparently the principal site of unhairing action. Treatment with certain salt solutions altered the

appearance of this layer and caused preliminary separation of the epidermis. Staining with crystal violet indicated that globular proteins are not an important constituent of this region but are subject to salt extraction. Metachromatic staining, said to be specific for acid mucopolysaccharides, failed to make the basement membrane visible but gave positive results in other locations, some of which were altered by salt treatment. Alcian blue helped to confirm these results.

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DISCUSSION

DR. JULIUS PFANNMULLER (Wallerstien Company, Inc.): We thank Dr. Cordon for a very interesting paper. I think Dr. Cordon's paper looks into the future. As far as I know, the tanners are quite dissatisfied with chemical

methods in unhairing hides and skins and are looking towards enzymatic methods. Whether such enzymatic methods will ever be realized on a large scale, we do not know. Of course, we all hope they will, because the problem of sewage disposal would be much easier. And this is a problem in practically every country.

As to the influence of salt on enzyme unhairing, the influence of salt on chemical unhairing of skins is known. We know that it dissolves certain globulins. Generally we might say that salt interferes in chemical unhairing of skins with lime. High salt concentration in enzymatic unhairing should also interfere. However, according to this paper, it seems that a small addition of salt aids enzymatic unhairing.

Now we have to see the practical side of enzymatic unhairing. Enzymatic unhairing in this country was carried out on a large scale on goatskins. But according to Dr. Cordon, the technical process was only carried out by first swelling the skins with caustic and then unhairing with enzymes.

I think Dr. Cordon carried out his unhairing experiments on skins that were not pretreated with any caustic materials. He did not use a pure single enzyme but rather enzyme mixtures. We probably could get more fundamental knowledge if we would work with pure enzymes. The best would be to use crystalline enzymes.

On the other hand, pure enzymes are very expensive, so enzyme mixtures will be used for technical unhairing. According to the findings of Dr. Cordon, there was a very big difference in unhairing skins that had been kept six months in cold storage. Now certainly, certain mechanical destruction of the structure of the skin caused by water crystallization should be expected. Salt showed no influence in unhairing hides after six months storage. Probably some mechanical destruction of the fiber structure allowed the enzyme to penetrate more easily.

I wish that some people here would tell me a little about enzymatic unhairing. I hear that it is now carried out in Europe with high concentrations of enzymes without previous swelling of the skin. They take hides where the salt is thoroughly washed out. They use practically no water. Later, for easier mechanical unhairing, they swell the hides with a little caustic. That is just about the opposite of what was formerly done in the enzymatic unhairing process—the one used in this country for unhairing goatskins.

DR. L. T. KREMZNER (General Foods Corp.): I would like to bring to the attention of the speaker some recent work reported by a Dr. Nickerson of Rutgers University and the Institute of Microbiology. He has reported on a microorganism which secretes keratinase, and this keratinase will, in a couple

of hours, dehair a hide. That is all that I know about it now, except that I do understand that there will be a commercial process in a short time—that is, the manufacture of the enzyme.

DR. CORDON: I know of Dr. Nickerson's work at Rutgers, having seen an abstract of a paper he presented about a year ago. I believe he described some keratinase enzymes. I wrote to him for some of his cultures, but he did not answer my letter.

We have in mind work along that line, to investigate certain purified enzymes to see their effects on specific components of the hide.

DR. PFANNMULLER: I might add something: We have two basicly different unhairing principles. Unhairing with the proteolytic enzymes undoubtedly is due to a proteolytic action. The keratinase unhairing action is probably due to a disulfide reductase enzyme, so this enzymatic action should be similar to that occurring in lime with sharpness. The disulfide reductase should break the disulfide links of the keratin, an action similar to that occurring in a chemical unhairing process. Disulfide reductases from an enzymatic standpoint are difficult to obtain and to determine exactly.

DR. H. G. TURLEY (Rohm & Haas, Philadelphia, Pa.): I can contribute nothing to the matter just discussed. However, on the subject of enzymatic unhairing, it looks like the pendulum is swinging back again. Forty years ago or so, there was great interest in that, and there was a certain amount of success achieved. Then I think we can safely say that it died out substantially.

My own feeling about the matter is that this type of process requires too much direction and study, and so forth, for the usual tanner. Times have changed, however, and we have better personnel who have more knowledge and more equipment, and now we also have the problem of sewage disposal, so enzyme unhairing has come up again. It has cropped up, I think, due to the work recently done in Great Britain. I can say it is sufficiently interesting in Europe that at the forthcoming meeting in Rome there will be about four papers on enzyme unhairing, and I think from about four different countries. One of them is concerned with enzyme unhairing using proteolytic complexes of streptomyces.

I saw one of Dr. Cordon's slides, and it showed the detachment of a portion of the epidermis. But curiously enough, the detachment did not extend into the hair roots. It is the same type of material. Does Dr. Cordon have any ideas about that?

Secondly, I think the sections would have been a little easier to see had there been better differentiation. They were rather heavily stained, and I don't know how much consideration has been given to the technique of the art. When that is treated carefully, we can usually see more.

DR. CORDON: As to the first question, Dr. Turley, the entire epidermis is loosened by salt-soaking including the sebaceous glands and hair follicles. This was shown on a slide before you came in.

As to the second question—thank you, we will try to improve our technique.

DR. PFANNMULLER: Do you think there could be two enzymatic unhairing methods—first, one with proteolytic enzyme systems, and another one similar to the chemical method with enzyme systems that break down disulfide links?

DR. TURLEY: It might be possible, although we do not know for sure too much about the chemistry in the lower layer of the epidermis—the germinating layer—that one might be eliminated. It would be a matter of dissolving keratin or some reduction of disulfide groups. But this might not be so in unhairing. It seemed not to be so in the old days with caustic soda. All we need is a straightforward proteolytic digestion. After what you have said, Dr. Pfannmuller, it pleases me to know that in Europe today there is a reversal of the old process—namely, not to swell the skins first. In some quarters that is thought to be bad, but that can be argued.

TITUS WEAVER (Ohio Leather Company): I cannot add anything as far as the enzyme unhairing is concerned, outside of the fact, for general information, that we have processed the skins that were enzymatically unhaired at Rutgers University by Dr. Nickerson. At present we are putting through the second set that he has unhaired. I will say that he has done a beautiful job of unhairing. I don't want to say anything about the quality of the leather as yet. But it has very good possibilities. The unhairing was complete.

Dr. PFANNMULLER: May I ask you what enzyme he used for unhairing? What was the origin of the enzyme?

MR. WEAVER: I don't know that. We are only processing the skins after he has unhaired them. These skins were unhaired at Rutgers University, and we processed the skins into finished leather.

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